

Chapter 1

Consideration of Combined Stress: A Crucial Paradigm for Improving Multiple Stress Tolerance in Plants

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1.1 Introduction

Food security is a major issue in the global policy agenda. In the next 40 years, demand for cereal production is predicted to increase by 60% as the population rises from the current 6.6 to 8.7 billion by the year 2050 (Bengtsson et al. 2006). In a world where population growth exceeds food supply (Malthus 1817), a second green revolution is necessary. But the challenges in overcoming the constraints in food production are complex. The ongoing change in climate mostly due to anthropogenic activities causes increases in carbon dioxide (CO₂) emissions (Peters et al. 2011), further exacerbating the agricultural land deterioration due to increasing temperature (Kissoudis et al. 2014). Increasing temperature in turn leads to higher evapotranspiration, drought intensification, and increasing soil salinization (Munns and Tester 2008; Zhao and Running 2010). Though the existing data on the impact of climate change on pathogen spread are inconclusive, evidence points to increased reproductive potential and geographic expansion leading to interactions with more hosts and new virulent pathogenic strains (Garrett et al. 2006). An analysis of the natural disasters that resulted in more than a billion dollars in the USA in the past three decades clearly shows that both the frequency and intensity of these events are increasing (Fig. 1.1). Hence, the chances of plants encountering new combination of stresses in the future are likely to be higher. It thus behooves upon plant scientists working on stress resistance to consider the combination of stresses that are likely to co-occur under field conditions.

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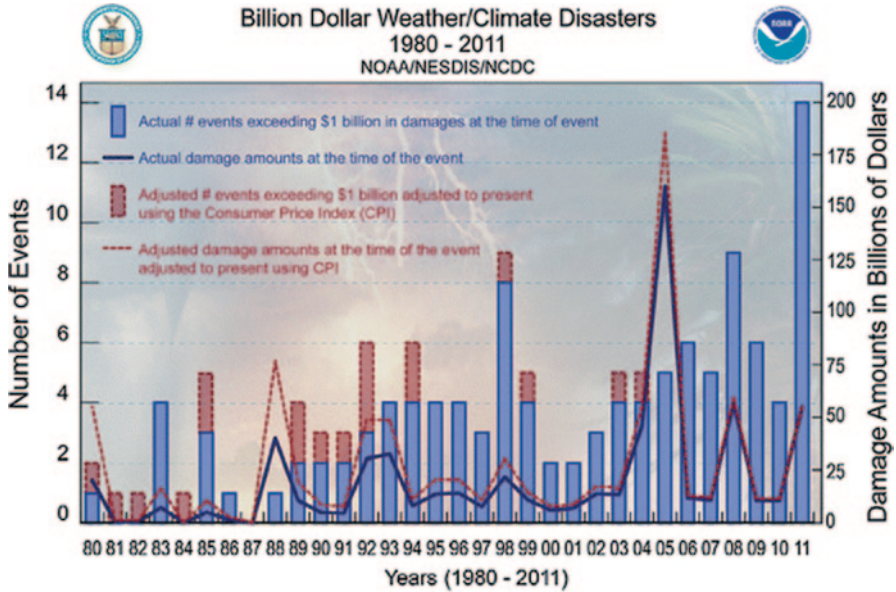


Fig. 1.1 The US billion-dollar weather and climate disaster time series from 1980 to 2011. (Adapted from reference Smith and Katz 2013)

1.2 Importance of Combined Stress

Literature is replete with studies on plant responses to stresses. PubMed search using keywords “stress” and “plants” in title and abstract field alone identified nearly 15,300 citations while “combined stress” and “plants” retrieved 480 citations. A closer inspection of the latter search revealed only around 180 original articles that actually dealt with the combination of two or more stresses in plants. A listing of primary research articles on combined stress in various plant species is given in Table 1.1.

The combined occurrence of drought and heat in the USA from 1980 to 2012 was shown to cause fivefold more damage when compared to drought alone (Fig. 1.2). Increase in global surface temperature is a major indicator of global warming (Van Vuuren et al. 2008). This rise in mean global temperature is attributed to increases in the greenhouse gases such as CO_2 and air pollutants such as ozone (O_3) that have been brought about by anthropogenic activities. For the first time in recorded history, the average level of CO_2 has topped 400 parts per million (ppm) for an entire month in April 2014 according to the Scripps Institution of Oceanography. Efforts to control CO_2 emissions on a global scale will be difficult to enforce given the political and economic implications surrounding such legislations. More than 400 ppm of CO_2 may thus be the new reality for crop plants in the future.

Table 1.1 Primary research studies of combined stresses in various plant species

Stress combination	Plant species (references)
Drought + heat	<i>Arabidopsis</i> (Koussevitzky et al. 2008; Rizhsky et al. 2004; Vile et al. 2012; Wolfe and Tonsor 2014), tobacco (Cvikrova et al. 2013; Rizhsky et al. 2002), wheat (Keles and Oncel 2002; Prasad et al. 2011; Rampino et al. 2012; Szucs et al. 2010; Yang et al. 2011), <i>Sorghum</i> (Johnson et al. 2014), <i>Carissa spinarum</i> (Zhang et al. 2010), lotus (Sainz et al. 2010), soybeans (Simon-Sarkadi et al. 2005), <i>Jatropha</i> (Silva et al. 2010); barley (Rollins et al. 2013); poplar (Centritto et al. 2011); prosopis (Delatorre et al. 2008)
Drought + chilling	Sugarcane (Sales et al. 2013), maize (Aroca 2003)
Drought + high light	<i>Arabidopsis</i> (Estavillo et al. 2011; Giraud et al. 2008), <i>Haberlea rhodopensis</i> (Georgieva et al. 2010), rice (Zhou et al. 2007), watermelon (Nanasato et al. 2005), pearl millet and <i>Sorghum</i> (Masojidek et al. 1991); <i>Nerium oleander</i> (Demmig et al. 1988)
Drought + heavy metals	Red maple (de Silva et al. 2012); <i>Populus cathayana</i> (Han et al. 2013); oak (Sardans and Penuelas 2007); <i>Stackhousia tryonii</i> (Bhatia et al. 2005)
Drought + ozone	Birch (Paakkonen et al. 1998), beech (Nunn et al. 2007), <i>Medicago truncatula</i> (Iyer et al. 2013), <i>Quercus</i> (Alonso et al. 2014), poplar (Bohler et al. 2013), <i>Dactylis glomerata</i> , and <i>Ranunculus acris</i> (Wagg et al. 2012); wheat (Biswas and Jiang 2011; Herbiniger et al. 2002), spruce (Karlsson et al. 1997; Kivimaenpaa et al. 2003); <i>Pinus halepensis</i> (Manes et al. 2001; Fontaine et al. 2003)
Drought + salinity	Barley (Ahmed et al. 2013a, b, c); <i>Sesuvium portulacastrum</i> (Slama et al. 2008)
Drought + soil compaction	Tobacco (Alameda et al. 2012)
Drought + nutrients	Maize (Kandianis et al. 2013; Makumburage and Stapleton 2011); wheat (Wei et al. 2013); potato (Germ et al. 2007)
Drought + UV	Maize (Makumburage et al. 2013); wheat (Feng et al. 2007; Zhao et al. 2009); <i>Arabidopsis</i> (Comont et al. 2012; Schmidt et al. 2000); barley (Bandurska et al. 2012); peas (Nogues et al. 1998); <i>Populus cathayana</i> (Lu 2009); willows (Turtola 2006); soybeans (Sullivan and Teramura 1990)
Drought + high CO ₂	Potato (Barnaby et al. 2014); maize (Sicher and Barnaby 2012); <i>Phaseolus vulgaris</i> (Medeiros and Ward 2013); <i>Viguiera discolor</i> (Oliveira et al. 2013); eucalyptus (Crous et al. 2012; Duursma et al. 2011; Lewis et al. 2013; Zeppel et al. 2011); maize and sorghum (Allen et al. 2011; Kakani et al. 2011; Leakey et al. 2006); pepper (del Amor et al. 2010); populus (Bobich et al. 2010); cucumber (Li et al. 2008); oak and pine (Schwanz et al. 1996)
Drought + pathogens/pest	<i>Arabidopsis</i> (Atkinson et al. 2013; Anderson et al. 2004); tobacco (Ramegowda et al. 2013); rice (Campo et al. 2012); <i>Alnus fruticosa</i> (Rohrs-Richey et al. 2011); beet and rice (Xu et al. 2008)
Salinity + heat	Tomato (Rivero et al. 2014); poplar (Behnke et al. 2013); <i>Artemisia</i> (Wen et al. 2005) <i>Swietenia macrophylla</i> (Rahman et al. 2013)

Table 1.1 (continued)

Stress combination	Plant species (references)
Salinity + ozone	Alfalfa (Maggio et al. 2009); chickpea (Welfare et al. 2002); rice (Welfare et al. 1996); wheat (Zheng et al. 2012)
Salinity + pathogens	Rice (Xiong and Yang 2003)
Salinity + nutrients	Barley (Talbi Zribi et al. 2011); <i>Hordeum maritimum</i> (Talbi Zribi et al. 2012); spinach (Kaya et al. 2001); soybeans (Grattan and Maas 1988); peanuts (Silberbush and Ben-Asher 1989); <i>Crithmum maritimum</i> (Labidi et al. 2011); broccoli (del Carmen Martinez-Ballesta 2008)
Salinity + high CO ₂	<i>Arabidopsis</i> (Kanani et al. 2010); <i>Spartina maritima</i> (Mateos-Naranjo et al. 2010a); barley (Perez-Lopez et al. 2009, 2012); pepino (Chen et al. 1999); melon (Mavrogianopoulos et al. 1999); citrus (Garcia-Sanchez et al. 2006); olive (Melgar et al. 2008); aster (Geissler et al. 2009, 2010); tomato (Takagi et al. 2009); <i>Spartina densiflora</i> (Mateos-Naranjo et al. 2010b)
Heat + ozone	Birch (Kasurinen et al. 2012; Maenpaa et al. 2011; Riikonen et al. 2009, 2013); spruce (Riikonen et al. 2012); populus (Hartikainen et al. 2009); bean (Albertine and Manning 2009); radish (Kleier et al. 2001)
Heat + light	Sunflower (Hewezi et al. 2008); <i>Brassica</i> (Diaz et al. 2007); oats (Quiles 2006); seagrass (York et al. 2013); apple (Chen et al. 2008); grapes (Greer and Weedon 2012); <i>Arabidopsis</i> (Burgos et al. 2011; Lokhande et al. 2003; Vasseur et al. 2011); <i>Dunaliella salina</i> (Haghjou et al. 2009); <i>Phragmites australis</i> (Loreto et al. 2006); wheat (Monneveux et al. 2003); spruce (Mahoney et al. 1998)
Heat + UV	Wheat (Zheng et al. 2011); cucumber (Caldwell 1994)
Heat + high CO ₂	Tomato (Li et al. 2014b); Kentucky bluegrass (Song et al. 2014); aspen (Sun et al. 2013); soybeans (Sicher 2013); rice (Madan et al. 2012); eucalyptus (Loveys et al. 2006); bell pepper (Aloni et al. 2001; Karni and Aloni 2002); <i>Abutilon theophrasti</i> (Ziska 2001); cotton and tobacco (Crafts-Brandner and Salvucci 2000)
Temperature + pathogens	<i>Arabidopsis</i> (Szittyta et al. 2003; Yang and Hau 2004; Zhu et al. 2010); tomato (de Jong et al. 2002)
Ozone + high CO ₂	Soybeans (Ainsworth et al. 2008; Gillespie et al. 2012); populus (Kets et al. 2010); wheat (Mishra et al. 2013)
Ozone + UV	Linseed (Tripathi and Agrawal 2013a, b); birch (Pliura et al. 2008); <i>Elymus athericus</i> (van de Staaij et al. 1997)
Ozone + pathogens	Tobacco (Ye et al. 2012); soybeans (Bilgin et al. 2008); Beech and spruce (Luedemann et al. 2005)
Chilling + high light	Tomato (Wang et al. 2008); cotton (Kornyejev et al. 2001; Payton et al. 2001)
Chilling + pathogens	<i>Arabidopsis</i> (Yang et al. 2010)
UV + heavy metals	<i>Brassica campestris</i> (Shukla et al. 2008); <i>Pisum sativum</i> (Srivastava et al. 2012)
UV + pathogens	<i>Arabidopsis</i> (Kunz et al. 2006); tea (Gunasekera et al. 1997); cabbage (Brown et al. 2001); tobacco (Yalpani et al. 1994)

Table 1.1 (continued)

Stress combination	Plant species (references)
High CO ₂ + high light	<i>Chlorella</i> (Kozłowska-Szerenos et al. 2004)
Nutrient + pathogens	<i>Arabidopsis</i> (Ammann et al. 2008)
Drought + heat + high light	<i>Hibiscus</i> (Munoz and Quiles 2013); <i>Rosa meilandina</i> (Paredes and Quiles 2013); wheat (Sharma and Singhal 1993)
Drought + high light + UV	<i>Arabidopsis</i> (Poulson et al. 2006)
Drought + heat + virus	<i>Arabidopsis</i> (Prasch and Sonnewald 2013)
CO ₂ + temperature + UV	Cowpea (Singh et al. 2010); soybeans (Koti et al. 2005); birch (Lavola et al. 2013)
Ozone + light	<i>Trifolium subterraneum</i> (Vollnes et al. 2009)
CO ₂ + temperature + drought	Eucalyptus (Rodén and Ball 1996)
UV + nutrients	<i>Vigna radiata</i> (Agrawal et al. 2006); wheat (Shukla et al. 2002)
CO ₂ + ozone + insects	Soybeans (Casteel et al. 2008)
CO ₂ + temperature + insects	Soybeans (Niziolek et al. 2013)

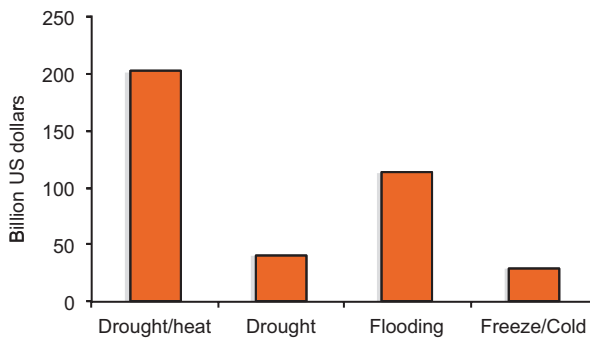


Fig. 1.2 A meta-analysis of naturally occurring disasters in the USA. Losses due to weather-related disasters (excluding tornadoes, hurricanes, and wildfires) occurring between 1980 and 2011 that exceeded more than a billion dollars were included in this analysis. Damage costs were normalized to the 2013 US dollar value. Raw data for this analysis were from reference (Smith and Katz 2013)

Here is an example to illustrate the importance of considering more than a single stress. A recent study indicated that rising CO₂ levels increased the estimated yield levels of soybeans during 2002–2006 by 4.34, 7.57, and 5.10%, in the USA, Brazil, and China, respectively (Sakurai et al. 2014). However, there are other studies using the free-air concentration enrichment (FACE) technology that consider the increasing levels of ozone, the most abundant air pollutant that will negate the fertilizing effects of CO₂ and predict a less-than-expected yield due to the increasing levels of CO₂ (Long et al. 2005, 2006).

1.3 Which Combination of Stresses to Study?

This begs the question which combination of stresses to study. As indicated earlier, plants are continually challenged by diverse array of biotic and abiotic agents from seed germination to senescence. We can envisage considering those stresses that are most likely to co-occur under field conditions and whose combined impact can adversely affect the final yield.

Stress Matrix Approach Mittler and coworkers have advocated the use of a stress matrix showing different combinations of potential environmental stresses that can affect crops. The use of colors to indicate potential positive and negative interactions provides a visually appealing schema for depicting combined stresses (Fig. 1.3). It

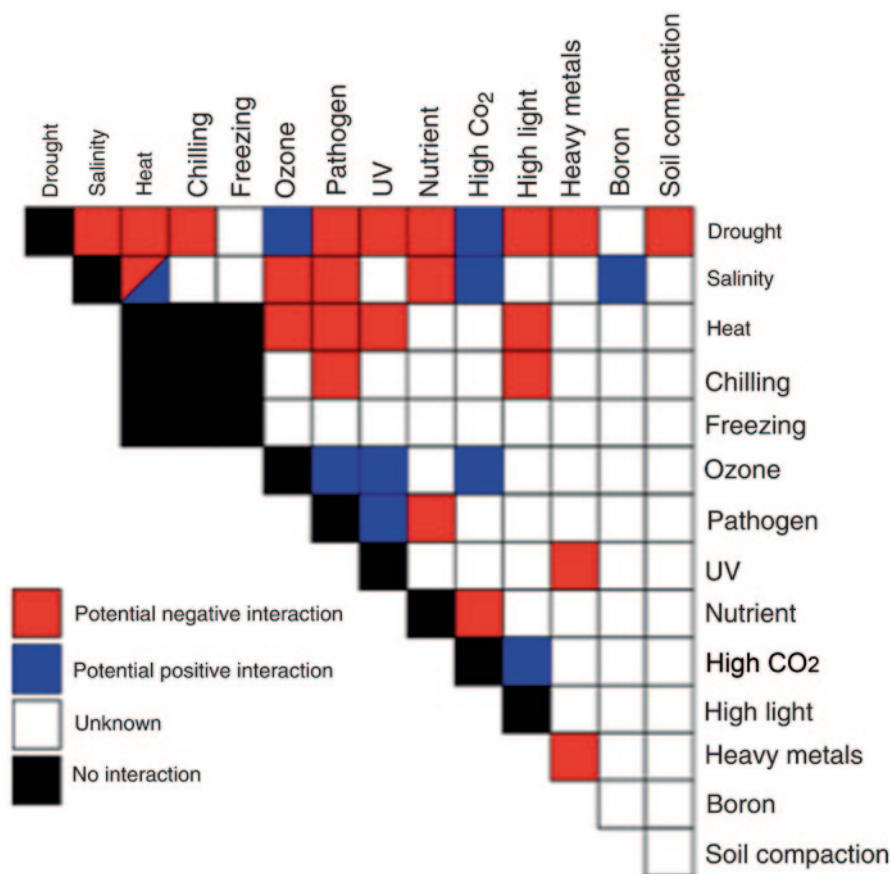


Fig. 1.3 The stress matrix. Different combinations of potential environmental stresses that can affect crops in the field are shown in the form of a matrix. The matrix is color-coded to indicate stress combinations that were studied with a range of crops and their overall effect on plant growth and yield. References for these studies are given in the text and in Table 1.1. (Adapted from Suzuki et al. (2014) and modified from Mittler (2006))

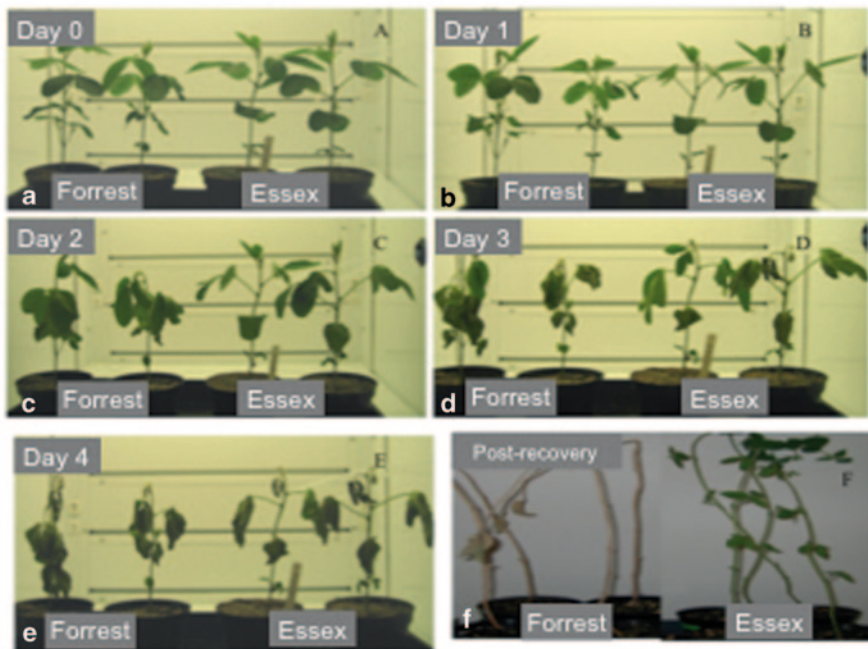


Fig. 1.4 Combined ozone and drought stress in two soybean cultivars. Cultivar Forrest (sensitive to ozone, sensitive to heat) and Essex (tolerant to ozone, tolerant to heat) were simultaneously exposed to 75–100 ppb of ozone and higher temperature of 37°C for 3 h a day for 3 consecutive days. Photographs were taken at the end of the combined ozone and heat treatment on day 1 (A), day 2 (B), day 3 (C), 24 h after the end of the treatment (E), and 10 days of post-recovery in a growth chamber (F)

should be noted that this is a vast oversimplification of the complexity involved in combined stress scenarios. For example, *Medicago truncatula* cultivar Jemalong is sensitive to ozone and drought when the stresses were applied singly (Puckette et al. 2007). The combined application of drought and ozone in Jemalong evoked a very different transcriptome and metabolic response that manifested as a stress-tolerant phenotype (Iyer 2013). To test whether this observation can be extended to other legumes, we used the soybean cultivar Forrest that is sensitive to drought and ozone when applied singly. The combined application of drought and ozone for a period of 3 days was detrimental for Forrest plants (Fig. 1.4). Thus, based on single-case studies, it is naïve to interpret the interactions between stress combinations as positive or negative. Biswas and Jiang (2011) reported that, under conditions of combined ozone and drought stress, the ozone-sensitive modern winter wheat cultivar (*Triticum aestivum* L. cv. Xiaoyan 22) improved its tolerance against ozone, while the ozone-tolerant primitive wheat (*Turgidum* ssp. durum) lost ozone tolerance. Crops show wide variability in their phenotypic responses to stresses and this includes both the intra- and inter-specific variation (Biswas et al. 2008; Brosche et al. 2010).

Furthermore, it has been shown that the order in which the stress combinations are applied may evoke a different response. An early drought could lead to a decrease in stomatal conductance and a subsequent protection against a later ozone exposure while the appearance of drought during preexisting ozone stress would suffer under the appearing sluggishness of stomata, initially caused by ozone (Paoletti and Grulke 2010).

Plants can show varied responses to stresses depending on their developmental stage. This adds an additional layer of complexity in the analysis of plant stress studies. If a field is affected by stress at a very early stage of development (e.g., seedling stage), a farmer may be able to undertake second planting and still recover his losses. On the other hand, a severe stress in field during the reproductive stage of development will not be amenable for such amends. It has been reported that most plants of agronomic importance are gullible to abiotic stresses during reproductive stages with detrimental consequences to the yield (Barnabas et al. 2008). Interestingly, the consequences to yield in response to stresses are not considered in most studies involving model plants like *Arabidopsis*. The usefulness of model plants for understanding plant stress responses can be greatly increased by assessing impact of stress on seed yield and seed quality. From an agronomic perspective, the most important aspect of plant stress interactions will be to understand its impact on the final yield.

1.4 Omics of Combined Stress

A detailed review of the transcriptome studies on combined stresses in plants has been reported (Jambunathan et al. 2010). A few proteomic studies on the combined stresses have been reported. This includes drought and ozone in poplar (Bohler et al. 2013), drought, and heat in *Arabidopsis*, barley, *Carissa spinarum* (Koussevitzky et al. 2008; Rollins et al. 2013; Zhang et al. 2010), toxic compounds like mercury and salinity in *Suaeda salsa* (Liu et al. 2013), high temperature and humidity in *Portulaca oleracea* (Yang et al. 2012). Interestingly, transcriptomic and proteomic analysis of several different combined stresses in several different plant species converges on the antioxidant defense machinery as a key pathway. The observed higher antioxidant capacity and/or lower accumulation of the reactive oxygen species (ROS) seems to be a mechanism operative in plants tolerant to combined stresses (Iyer et al. 2013; Koussevitzky et al. 2008; Ahmed et al. 2013b; Perez-Lopez et al. 2009; Rivero et al. 2014; Sales et al. 2013). Omics approaches have also shown that there are unique transcription factors, hormone-responsive genes and osmolytes that are differentially expressed in response to different combined stresses (Iyer et al. 2013; Atkinson et al. 2013; Rasmussen et al. 2013; Rizhsky et al. 2004). An apparent gap in the knowledge is the lack of information on posttranscriptional gene regulation by microRNAs in response to combined stresses. In fact, a comprehensive analysis of transcriptome, proteome, metabolome, and miRNome even in response to a single stress has not been reported. Such integrated omics

studies of combined stresses imposed during reproductive stages of crop development are warranted.

DNA cytosine methylation and histone modifications such as methylation and acetylation affect transcription especially in response to changes in environment (Mirouze and Paszkowski 2011). Epigenetic modifications involving chromatin-regulated gene activation govern priming responses (Conrath 2011) and widespread alterations in DNA methylation have been reported in response to biotic and abiotic stresses (Bilichak et al. 2012; Downen et al. 2012). The knowledge of epigenetic modifications in the wake of combined stresses is relatively unknown and is worthy of further investigations. It has been speculated that epigenetic modifications in response to a stress may predispose plants to a subsequent stress by either sensitizing or desensitizing. Such acclimation/predisposition may provide a novel avenue for preparing seeds for stressful environments (Kissoudis et al. 2014).

1.5 Phenotypic Responses to Stresses

From an agronomic point of view, the definition of plant sensitivity to stresses can be misleading. For example, crops can be sensitive to ozone with reference to visible foliar damage at early stages of growth but may not have a net impact on the grain yield during harvest. In rice and wheat, plants with least visible foliar symptoms showed maximum yield losses (Picchi et al. 2010; Sawada and Kohno 2009) and this was explained on the basis of stomatal closure response. Cultivars in which ozone causes stomatal closure prevent the influx of ozone and reduce the extent of foliar injury. Thus, based on the damage to leaves, these cultivars are resistant to ozone. However, prolonged stomatal closure affects carbon fixation and in turn the amount of assimilates required for grain filling. Thus, with reference to yield these cultivars are ozone sensitive. Other mechanisms for the negative effect of ozone could be due to the reduction of new growth (McKee and Long 2001), reduced root biomass (Grantz et al. 2006), reduced phloem translocation efficiency, or reduced carbon partitioning to grains over synthesis of protective chemicals (Betzberger et al. 2010).

It is important to understand the differences between sensitive and resistant responses that can differ depending on the stress. Let us consider the example of ozone exposure. The visible injury symptoms due to ozone are mostly assessed by damage to foliage. In sensitive plants, they appear as small chlorotic or necrotic lesions on leaves that can coalesce into larger patches of injured area, and such leaves usually senesce early. This reduces the effective biomass that in turn will take a toll on crop yields (Wilkinson et al. 2012). The same necrotic lesions on the foliage in response to avirulent pathogen infections are termed as hypersensitive response and the plant is considered to be resistant to the pathogen. The characterization of the same phenotype as being resistant with respect to one stress and as sensitive response to another stress is important to bear in mind while considering the combination of biotic and abiotic stresses.

1.6 Contrasts Between Laboratory and Field Studies

In several recent reviews, the limitations of single stress studies in controlled conditions compared to field conditions have been examined (Mittler and Blumwald 2010; Suzuki et al. 2014). The study of combined stresses in the laboratory is advocated so that the molecular pathways for tolerance to stresses that prevail under field condition can be identified. Most of the studies on combined stresses so far have been conducted under growth chamber or greenhouse conditions (Suzuki et al. 2014). Here, we have contrasted the combined stress studies in laboratory conditions versus the field conditions (Fig. 1.5). Combined stresses dealing with edaphic factors can be conducted effectively in greenhouse conditions. This includes the combinations of drought and nutrients, drought and salinity, drought and soil pathogen/pests such as nematodes. Combined stress experiments that involve interactions between climate change factors including CO₂, ozone, and temperature extremes (heat or cold) are ideal for growth-chamber studies. But the main constraint here is the number of large-sized plants that can be accommodated in such chambers. If greenhouse space and infrastructure for regulating gaseous mixtures (for example, CO₂ and ozone) are available, it provides an ideal platform for conducting controlled combined stress analysis of climate change variables and edaphic factors. Several reviews have examined the advantages and disadvantages of open-top chambers (OTCs), FACE systems, and screen-aided CO₂ control (SACC; Ainsworth et al. 2008; Li et al. 2007). Though FACE and OTCs provides an opportunity to examine the impact of climate change factors in actual field environment, it will be hard to use these facilities in combined stress scenarios such as drought or temperature stress. Rainout shelters can be constructed for studying drought in combination with other climate change factors in a FACE but may be expensive.



Growth chamber
Controlled environmental conditions. Studies on stress treatment combinations are easy to conduct. No interactions with other weed plants, or insect pests

Uniform soil but limited soil volume

Most suitable for small sized plants like Arabidopsis, brachypodium, foxtail millet.



Green house
Controlled environmental conditions. Only some stress treatment combinations are easy to conduct. No interactions with other weed plants, some insect pests possible

Uniform soil but limited soil volume – can be improved by using large sized pots

Can accommodate more number of plants compared to a growth chamber. Suitable for larger plants like soybeans, wheat, corn



Field studies
Heterogeneous environmental conditions. Plants are exposed to multiple stresses from germination to maturity. Constant interactions with weeds, pests, and vagaries of nature

Soil physical properties maybe heterogeneous but soil volume is not limiting

Can accommodate large number of plants for providing robust assessment of phenotypes in any crop plants

Fig. 1.5 Comparisons between growth chamber, green house, and field studies for analyzing the effects of combined stresses in plants

1.7 Advances in Phenomics

Following the enormous advances in the sequencing technologies, it has now become routine to sequence large collections of accessions or mapping populations in a plant species (Lam et al. 2010; Li et al. 2014a; Weigel and Mott 2009). The major bottleneck currently in utilizing the genome sequence deluge is the ability to procure reliable phenotype data. Over the past decade field, phenotyping has made rapid strides by utilizing remote-sensing technologies for crop monitoring (Furbank and Tester 2011). The field of phenomics described as a “high-throughput plant physiology” makes use of noninvasive imaging, infrared thermography, spectroscopy, robotics, image analysis, and high-performance computing. Several successful phenotyping screens for single stresses such as drought, UVB have been reported in model plant systems (Jansen et al. 2010; Woo et al. 2008) as well as in crop plants (Chapuis et al. 2012; Honsdorf et al. 2014; Sirault et al. 2009).

For UV stress and temperature extremes, the photosynthetic light-harvesting apparatus is often the first site of damage. UV stress can result in oxidative damage to the photosystems, perceived as a loss of efficiency of light harvesting, that can be exploited as a screening tool for tolerance to UVB exposure (Jansen et al. 2010). In the case of temperature extremes, the effects on photosynthesis and even changes in membrane lipid properties can lead to immediate effects on chlorophyll fluorescence (Armond et al. 1980).

Digital imaging in visible wavelength regions provides information on plant size, and also on the color of the plants. This information enables the quantification of senescence arising from nutrient deficiencies or toxicities, or pathogen infections. Germanium, a toxic analog of boron, was tested in a mapping population of barley to identify a Quantitative Trait Loci (QTL) at the same locus as previously identified for boron tolerance using a visual score of symptoms (Schnurbusch et al. 2010).

Near-surface reflectance spectroscopy was used to monitor the leaf nitrogen and chlorophyll content and epoxidation state of xanthophyll cycle pigments in field-grown soybean plants exposed to ozone (Ainsworth et al. 2014). This study shows that the leaf optical properties can be monitored using remote-sensing techniques to assess ozone damage and provide a promising tool for elucidating ozone tolerance in plants.

The examples mentioned above demonstrate the utility of the phenomics tools for precisely monitoring the physiological impacts of single stresses such as drought, salinity, nutrient deficiency, and air pollutants. It is conceivable that these tools will be harnessed for the analysis of combined stresses in the future.

1.8 Strategies for Improving Tolerance to Combined Stresses

Two major strategies can be envisaged for improving the tolerance to combined stresses (Fig. 1.6). First strategy involves the meta-analysis of whole genome expression studies in response to various biotic and abiotic stresses that can be

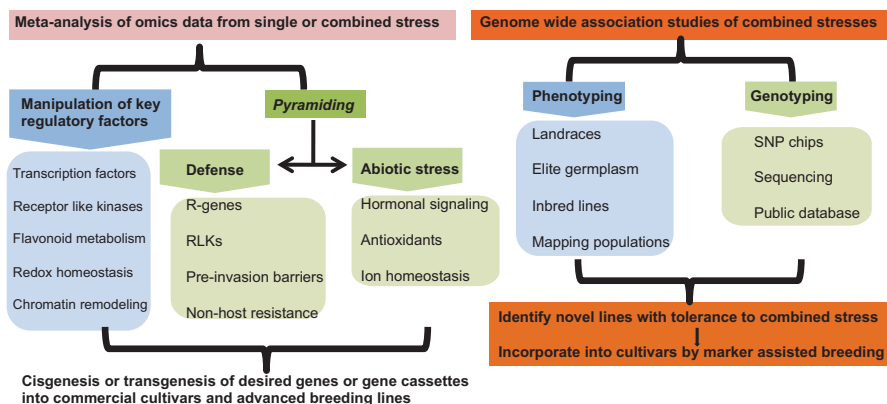


Fig. 1.6 Strategies for building tolerance to combined stresses in plants. A compendium approach for identifying key regulatory factors or by pyramiding key genes important in co-occurring stress scenarios that can be transferred into desired cultivars by genetic engineering. Another strategy will be to use genome-wide association mapping to identify novel germplasm containing alleles favorable for imparting tolerance to combined stresses and use naturally occurring variation for developing cultivars with improved resistance to multiple stresses via marker-assisted breeding

accessed through programs like Genevestigator (Zimmermann et al. 2004). Recent advances in computational tools such as co-expression modules and machine-learning approaches provide novel means for identifying the candidate genes for engineering broad-spectrum resistance based on gene expression data (Shaik and Ramakrishna 2013, 2014). Genetic components that potentially regulate the resistance to multiple stresses will be utilized for developing transgenic crops. Examples of genes for this strategy include stress-inducible transcription factors, receptor-like kinases, flavonoid metabolism, redox homeostasis, and chromatin modifications.

The same meta-analysis strategy can be adapted for gene pyramiding that has been successfully deployed for resistance to various plant pathogens (Joshi and Nayak 2010). In the case of combined biotic and abiotic stresses, the pyramided genes can be defense genes such as R-genes, pre-invasion defenses (such as callose deposition), nonhost resistance genes in combination with genes in the hormone signaling pathways, antioxidant defenses, or ion homeostasis (Fig. 1.6; Kissoudis et al. 2014).

A second strategy for improving plant tolerance to combined stresses involves the screening of large collections of germplasm in conjunction with genome-wide association mapping (Huang and Han 2014). In recent years, genotyping data for large collections of crop germplasms are becoming available in the public domain (Hao et al. 2012; Li et al. 2013; Song et al. 2013; Yu and Buckler 2006; Zhang et al. 2014). A reliable phenotypic evaluation of germplasm to various stress combinations of interest can be performed. The genotypic information from public domain can be exploited to precisely identify genomic regions associated with the traits of interest. The recent assembly and characterization of association mapping panels in various crop plants, development of improved statistical methods, user-friendly

tools for association mapping (e.g., GWAPP for *Arabidopsis*; TASSEL) and successful association of candidate genes have begun to realize the power of candidate-gene association mapping.

1.9 Conclusions/Perspectives

Studies of stress combinations that naturally occur under field conditions must be a priority for researchers working on abiotic and biotic stresses. Studies of such combined stresses should exploit the naturally occurring variation in the germplasm of crop plants to identify novel sources of resistance or tolerance. While imposing stress combinations, it is important to consider the plant developmental stages that can have the most detrimental agronomic consequences and conduct surveys of germplasm during these critical stages. Phenomic screening using noninvasive high-throughput phenotyping platforms will provide a wide spectrum of observations that span metabolic, physiological, and biochemical parameters. Though the initial costs are high for these setups, the long-term benefits are beyond comparison. Finally, integrating data from multiple omics platforms in conjunction with the phenotyping data will provide a cogent view of the responses to combined stresses in different genotypes. This is crucial for identifying the elite germplasm that can tolerate multiple stresses and provide maximum yields.

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